

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A16K 31/56, 31/365, G01N 33/53, 33/566	A1	(11) International Publication Number: WO 96/36230 (43) International Publication Date: 21 November 1996 (21.11.96)
(21) International Application Number: PCT/US96/03865 (22) International Filing Date: 17 April 1996 (17.04.96) (30) Priority Data: 08/442,464 16 May 1995 (16.05.95) US (60) Parent Application or Grant (63) Related by Continuation US 08/442,464 (CON) Filed on 16 May 1995 (16.05.95) (71) Applicant (for all designated States except US): THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, La Jolla, CA 92037 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): EVANS, Ronald, M. [US/US]; 1471 Cottontail Lane, La Jolla, CA 92037 (US). FORMAN, Barry, M. [US/US]; Apartment 299, 8568 Via La Jolla, La Jolla, CA 92037 (US). (74) Agent: REITER, Stephen, E.; Pretty, Schroeder, Brueggemann & Clark, Suite 2000, 444 South Flower Street, Los Angeles, CA 90071 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: MODULATORS FOR NEW MEMBERS OF THE STEROID/THYROID SUPERFAMILY OF RECEPTORS		
(57) Abstract <p>In accordance with the present invention, there are provided modulators for orphan member(s) of the steroid/thyroid superfamily of receptors which is related to the previously described CAR-α. Thus, compounds of the general class of androstans have been identified as modulators for a newly discovered isoform of CAR. Compounds discovered in accordance with the present invention can be employed in a variety of applications, e.g., for the modulation of processes mediated by an isoform of CAR or CAR-like species, to increase the libido of a subject (especially a subject undergoing therapy using a 5α-reductase inhibitor), in a screening assay for the presence of receptors involved in the modulation of libido, and the like. Also provided in accordance with the present invention are methods for the identification of compounds which modulate processes mediated by an isoform of CAR or CAR-like species, as well as novel compositions derived therefrom.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

Modulators for New Members of the
Steroid/Thyroid Superfamily of Receptors

FIELD OF THE INVENTION

The present invention relates to intracellular receptors, and modulators therefor. In a particular aspect, the present invention relates to methods for the
5 identification of compounds which function as modulators (or precursors thereof) for specific members of the intracellular receptor family. In other aspects, the present invention relates to various uses for the compounds so identified.

10

BACKGROUND OF THE INVENTION

A central problem in eukaryotic molecular biology continues to be the elucidation of molecules and mechanisms that mediate specific gene regulation. As part of the scientific attack on this problem, a great deal of work has
15 been done in efforts to identify modulators (i.e., endogenous or exogenous inducers and/or repressors) which are capable of mediating specific gene regulation.

Although much remains to be learned about the specifics of gene regulation, it is known that ligands
20 modulate gene transcription by acting in concert with intracellular components, including intracellular receptors and discrete DNA sequences known as hormone response elements (HREs).

As additional members of the steroid/thyroid
25 superfamily of receptors are identified, the search for endogenous or exogenous inducers and/or repressors for such newly discovered receptors has become an important part of the effort to learn about the specifics of gene regulation.

The identification of compounds which directly or indirectly interact with intracellular receptors, and thereby affect transcription of hormone-responsive genes, would be of significant value, e.g., for therapeutic applications.

Additional novel intracellular receptors (i.e., members of the steroid/thyroid superfamily of receptors) continue to be identified. Frequently, however, the primary ligand(s) for these novel receptors can not readily be identified. Accordingly, the identification of ligands and/or modulators for such receptors is of great value.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified modulators for orphan member(s) of the steroid/thyroid superfamily of receptors which is related to the previously described constitutively active receptor-alpha (CAR- α ; also known as "MB-67," see Baes et al., in Mol. and Cell. Biology 14:1544-1552 (1994)). Thus, compounds of the general class of androstans have been identified as modulators for a newly discovered isoform of CAR. Compounds discovered in accordance with the present invention can be employed in a variety of applications, e.g., for the modulation of processes mediated by an isoform of CAR or CAR-like species, to increase the libido of a subject (especially a subject undergoing therapy using a 5 α -reductase inhibitor), in a screening assay for the presence of receptors involved in the modulation of libido, and the like.

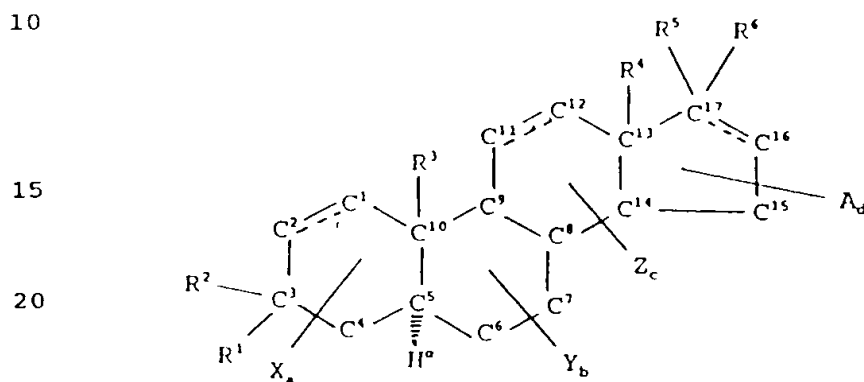
Also provided in accordance with the present invention are methods for the identification of compounds which modulate processes mediated by an isoform of CAR or CAR-like species, as well as novel compositions derived therefrom.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 illustrates the suppression of an isoform of CAR by 5 α -androstane derivatives.

DETAILED DESCRIPTION OF THE INVENTION

5 In accordance with the present invention, there are provided methods for modulating the activity of a CAR or CAR-like isoform, said method comprising administering an effective amount of a steroid-like compound having the structure I, as set forth below:



I

25 wherein:

- $R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and
 R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen,
 lower alkyl, acyl or trimethylsilyl;
 R^3 and R^4 are each independently hydrogen or lower
 30 alkyl;
 $R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6
 is absent when there is a double bond
 between C^{16} and C^{17} ;
 X , Y , Z and A are each independently selected
 35 from hydroxy, alkoxy (of a lower alkyl
 group), mercapto (of a lower alkyl group),
 halogen, trifluoromethyl, cyano, nitro,

amino, carboxyl, carbamate, sulfonyl, or
sulfonamide;

a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;

5 c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

As employed herein, the phrase "CAR or CAR-like isoform" refers to a member of the steroid/thyroid superfamily of receptors which is optionally constitutively
10 active, and has at least 75 % overall amino acid identity (up to 86 % sequence similarity) with the receptor set forth in SEQ ID NO:1 (CAR- α), at least 88 % amino acid identity (up to 91 % sequence similarity) in the DNA binding domain thereof, with respect to the DNA binding
15 domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid identity (up to 87 % sequence similarity) in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

20 As employed herein, the phrase "modulating the activity of a CAR or CAR-like isoform" refers to the ability of a modulator (e.g., a ligand or precursor thereof) for an isoform of CAR or a CAR-like species to induce expression of gene(s) maintained under hormone
25 expression control, or to repress expression of gene(s) maintained under such control.

As employed herein, the phrase "processes mediated by an isoform of CAR or a CAR-like species" refers to biological, physiological, endocrinological, and other
30 bodily processes which are mediated by receptor or receptor combinations which are responsive to natural or synthetic androstans. Modulation of such processes can be accomplished *in vitro* or *in vivo*. *In vivo* modulation can

be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

As employed herein, "lower alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; "alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; "substituted alkyl" refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), aryl, carboxyl, heterocyclic, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, and the like.

As employed herein, "acyl" refers to alkyl-carbonyl groups.

Presently preferred compounds employed in the practice of the present invention include those wherein R^1 of structure I is hydrogen and R^2 is α -OR (wherein R is as defined above, with R = hydrogen or acyl being especially preferred); compounds wherein R^3 of structure I is methyl; compounds wherein R^4 of structure I is methyl; compounds according to structure I wherein $R^5 = R^6 = O$; compounds wherein R^5 and R^6 of structure I are both hydrogen; compounds wherein R^6 of structure I is absent, and there is a double bond between C^{16} and C^{17} , and the like.

In accordance with another embodiment of the present invention, there are provided methods for the identification of compounds which modulate the activity of a CAR or CAR-like isoform (as defined herein), said method comprising:

contacting host cell(s) containing receptor-encoded DNA and a suitable hormone response element linked to reporter-encoded DNA with test compound, and

determining the effect of test compound on the level of expression of said reporter.

Optionally, the receptor-encoded DNA employed in the practice of the present invention will also encode one or more exogenous transactivation domains, such as, for example, the τ_1 or τ_2 transactivation domains described in United States Patent No. 5,217,867, which is incorporated by reference herein in its entirety.

Those of skill in the art can readily determine suitable response elements for use in the practice of the present invention, such as, for example, the response elements described in United States Patent No. 5,091,518 and PCT published application no. WO 92/16546, both of which are hereby incorporated by reference herein.

Identification methods according to the present invention involve the use of a functional bioassay system, wherein the CAR or CAR-like isoform (as defined herein) and a reporter plasmid are cultured in suitable host cells in the presence of test compound. Evidence of transcription (e.g., expression) of reporter gene is then monitored to determine the presence of an activated receptor-ligand complex. Accordingly, the functional bioassay system utilizes two plasmids: an "expression" plasmid and a "reporter" plasmid. The expression plasmid can be any plasmid which contains and is capable of expressing DNA encoding the CAR or CAR-like isoform receptor protein, in a suitable host cell. The reporter plasmid can be any plasmid which contains an operative hormone response element functionally linked to an operative reporter gene.

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β -gal), and the like. Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g.,

ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 5 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)]. The plasmids pGMCAT, pGHCAT, and the like, are examples of reporter plasmids which contain an operative hormone responsive promoter/enhancer element functionally linked to an 10 operative reporter gene, and can therefore be used in the above-described functional bioassay (see Example 1 for details on the preparation of these plasmids). In pGMCAT, the operative hormone responsive promoter/enhancer element is the MTV LTR; in pGHCAT it is the functional portion of 15 the growth hormone promoter. In both pGMCAT and GHCAAT the operative reporter gene is the bacterial gene for chloramphenicol acetyltransferase (CAT).

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", 20 the word "operative" means that the respective DNA sequences (represented by the terms "hormone response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means that after the two segments are linked, upon appropriate 25 activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "hormone response element" was "turned on" or otherwise activated.

In practicing the above-described functional 30 bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence and absence of a test compound to determine if the test compound is able to produce activation of the promoter 35 operatively linked to the hormone response element of the

reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include liver cell lines (e.g., Hep-G2), primary hepatocytes, adipocyte or pre-adipocyte cell lines (e.g., 3T3-L1 cells, 3T3-442-A cells, OB17 cells, and the like), as well as CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, NIH-3T3 cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1 derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound. "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with yet another embodiment of the present invention, there is provided a method to increase the libido of a subject, said method comprising inhibiting the activity of CAR or CAR-like isoforms (as defined

above). In a particular aspect the above-described method to increase libido can be carried out by administering to a subject a libido-enhancing amount of a steroid-like compound having the structure I, as described herein.

5 Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner.

10 In accordance with still another embodiment of the present invention, there are provided physiologically active composition(s) comprising a compound having the structure I, as described herein, in a suitable vehicle rendering said compound amenable to oral delivery,
15 transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, and the like.

 Pharmaceutical compositions of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the
20 like, wherein the resulting composition contains one or more of the compounds of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for enteral or parenteral applications. The active ingredient may be
25 compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used include glucose, lactose, gum acacia, gelatin,
30 mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary,

stabilizing, thickening and coloring agents and perfumes may be used. The active compound (i.e., compounds of structure I as described herein) is included in the pharmaceutical composition in an amount sufficient to
5 produce the desired effect upon the process or condition of diseases.

Pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily
10 suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may
15 contain one or more agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and
20 palatable preparations. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be, for example, (1) inert diluents such as calcium carbonate, lactose, calcium
25 phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, potato starch or alginic acid; (3) binding agents such as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The
30 tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl
35 distearate may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108;

4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

In some cases, formulations for oral use may be in the form of hard gelatin capsules wherein the active
5 ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive
10 oil.

The pharmaceutical compositions may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The
15 sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium.
20 For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate or the
25 like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Compounds contemplated for use in the practice of the present invention may also be administered in the form of suppositories for rectal administration of the drug.
30 These compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, it is up to the practitioner to determine a subject's response to treatment and vary the dosages accordingly.

Typical daily doses, in general, lie within the range of from about 0.5 μ g to about 10 mg per kg body weight, and, preferably within the range of from 50 μ g to 1 mg per kg body weight and can be administered up to four times daily. The daily IV dose lies within the range of from about 1 μ g to about 10 mg per kg body weight, and, preferably, within the range of from 10 μ g to 500 μ g per kg body weight.

In an alternate aspect of this embodiment of the present invention, compositions useful for ameliorating the libido-reducing effects of a 5 α -reductase inhibitor are provided. Such compositions comprise a libido-enhancing amount of a steroid-like compound having the structure I, as described herein, and a 5 α -reductase inhibitor.

Those of skill in the art can readily identify 5 α -reductase inhibitors suitable for use in the practice of the present invention. An example of a 5 α -reductase inhibitors contemplated for use in the practice of the present invention is finasteride (PROSCAR).

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner.

In accordance with yet another embodiment of the present invention, there is provided a method for ameliorating the libido-reducing effects of a 5 α -reductase

inhibitor, said method comprising co-administering, to a subject being treated with 5 α -reductase inhibitor(s), a libido-enhancing amount of a steroid-like compound having the structure I, as described herein.

5 In accordance with a still further embodiment of the present invention, there is provided a method of screening cells or cell extracts to determine the presence of receptors involved in the modulation of libido, said method comprising

10 contacting cells or cell extracts with a compound having the structure I, as described herein, and thereafter

identifying those cells or cell extracts which bind said compound.

The invention will now be described in greater
15 detail by reference to the following non-limiting examples.

Example 1

Preparation of reporter constructs

Various reporter constructs are used in the examples which follow. They are prepared as follows:

20 TK-LUC: The MTV-LTR promoter sequence is removed from the MTV-LUC plasmid described by Hollenberg and Evans in Cell 55:899-906 (1988) by *Hind*III and *Xho*I digest, and cloned with the *Hind*III-*Xho*I fragment of the Herpes simplex virus thymidine kinase gene promoter (-105 to +51 with
25 respect to the transcription start site, m, isolated from plasmid pBLCAT2, described by Luckow & Schutz in Nucleic Acids Res. 15:5490 (1987)) to generate parental construct TK-LUC.

5 pTK- β RARE_{1,2,3}-LUC: One, two or three copies of double-stranded beta-retinoic acid response element (β RARE) oligonucleotides, comprising a direct repeat of two half sites separated by a spacer of five nucleotides, wherein each half site comprises the sequence

N_x -RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

10 each N is independently selected from A, T, C, or G;

M is selected from A or C; and

x falls in the range of 0 up to 5;

15 with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-, is cloned upstream of the TK promoter of TK-LUC at the *HindIII* site.

20 Alternatively, response elements having a similar structure to that set forth above, except having a spacer of only four nucleotides, can be used. Thus, response elements comprising a direct repeat of two half sites separated by a spacer of four nucleotides, wherein each half site comprises the sequence

25 N_x -RGBNNM-,

as described above, can be used in place of the β RARE described above.

30 CMX- β GAL: The coding sequence for the *E. coli* β -galactosidase gene is isolated from plasmid pCH110 [see Hall et al., J. Mol. Appl. Genet. 2:101-109 (1983)] by *HindIII* and *BamHI* digest, and cloned into pCMX eucaryotic expression vector [see Umesono et al., *supra*].

Example 2Screening for CAR or CAR-like isoformsA. With PCR-generated probe

A probe spanning the DNA-binding domain of the
5 CAR-encoding DNA described by Baes et al. (Mol. and Cell.
Biol. 14:1544-1552 (1994); i.e., nucleic acid residues 303
to 545 of SEQ ID NO:1) is prepared by PCR. The probe is
labeled by the random-primer labeling method or by PCR
using ³²P nucleotides. The labeled probe is then used to
10 probe a lambda-gt11 mammalian liver cDNA library (e.g.,
mouse liver cDNA library or other readily available
library, such as are commercially available from Clontech
or Stratagene) to identify related receptors. The
hybridization mixture contains 35% formamide, 1X Denhart's,
15 5X SSPE (1X SSPE = 0.15 M NaCl, 10mM Na₂HPO₄, 1mM EDTA), 0.1%
SDS, 10% dextran sulfate, 100 µg/ml denatured salmon sperm
DNA and 10⁶ cpm of [³²P]-labelled probe. Duplicate
nitrocellulose filters are hybridized for 16h at 42°C,
washed once at 25°C for 15 min with 2X SSC (1X SSC=0.15 M
20 NaCl, 0.015 M sodium citrate), 0.1% SDS and then washed
twice at 55°C for 30 min. in 2X SSC, 0.1% SDS. The filters
are autoradiographed for 3 days at -70°C using an
intensifying screen.

After several rounds of screening, several
25 positive clones are obtained. Sequence analysis of at
least one of the positive clones indicates that this clone
encodes a novel member of the steroid/thyroid superfamily
of receptors, having approximately 75 % overall amino acid
identity with the receptor set forth in SEQ ID NO:1,
30 approximately 88 % amino acid identity in the DNA binding
domain thereof, with respect to the DNA binding domain of
the receptor set forth in SEQ ID NO:1, and approximately 74
% amino acid identity in the ligand binding domain thereof,

with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

If the initial clone isolated is a partial clone, then an insert of the above-identified positive clone
5 (labeled with ^{32}P) is also used as a probe to rescreen the same library or additional library(ies). Hybridization conditions for such rescreening comprise a hybridization mixture containing 50% formamide, 1X Denhart's, 5X SSPE, 0.1% SDS, 100 $\mu\text{g/ml}$ denatured salmon sperm DNA and 10^6 cpm
10 of [^{32}P]-labelled probe. Duplicate nitrocellulose filters are hybridized for 16h at 42°C , washed once at 60°C for 15 min with 0.1X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS and then washed twice at 60°C for 30 min. in 0.1X SSC, 0.1% SDS. The filters are
15 autoradiographed for 3 days at -70°C using an intensifying screen.

After several rounds of screening, several positive clones are obtained.

B. With synthetic oligonucleotides

20 A lambda-gt11 mammalian liver cDNA library is screened in duplicate with a ^{32}P -labeled synthetic oligonucleotide:

 TGYGARGGNTGYAARGGNTC TTT (SEQ ID NO:3),
under low-stringency conditions (i.e., 1M NaCl/0.05mM
25 Tris-HCl, pH 8.0/5mM EDTA/150 units of heparin per ml/0.05%, sodium pyrophosphate/100 μg of yeast RNA per ml/0.1% (wt/vol) NaDodSO₄ at 46°C) and washed at high stringency, as described by Burglin et al., in Nature 341:239-243 (1989). In the above oligonucleotide, Y is
30 selected from C or T, R is selected from A or G, and N is any one of A, G, C or T. Thus, the oligonucleotide employed is a mixture of all possible DNA sequences encoding the amino acid sequence:

CEGCKGFF (SEQ ID NO:4),

wherein each letter above is the conventional single letter abbreviation for amino acid residues, i.e., C is cysteine, E is glutamic acid, G is glycine, K is lysine and F is phenylalanine.

Example 3

Screening assay for modulators of CAR or CAR-like isoforms

CV-1 cells are co-transfected with a vector encoding the CAR isoform isolated as described in Example 2 (incorporated into a CMV-driven expression vector), and pTK- β RARE-LUC at a ratio of about 100 ng of receptor-encoding DNA per 10^5 cells. The usual amounts of DNA per 10^5 cells are 100 ng of CDM8-CAR, 300 ng of pTK- β RARE-LUC, and 500 ng of CMX- β GAL. Typically, transfections are performed in triplicate. The plates are then incubated for 2-3 hours at 37°C.

The cells are washed with fresh medium. Fresh medium containing one concentration of a serial dilution of agonist is added to each well. A typical agonist dilution series extends from 10^{-5} M through 10^{-11} M. A solvent control is performed for each agonist. The cells are incubated at 37°C for 1-2 days.

The cells are rinsed twice with buffered saline solution. Subsequently, cells are lysed, *in situ*, by adding 200 μ l of lysis buffer. After 30 minutes incubation at room temperature, 40 μ l aliquots of cell lysate are transferred to 96-well plates for luciferase reporter gene assays and β -galactosidase transfection controls [see Heyman et al., Cell 68:397-406 (1992)].

The data are expressed as relative light units (RLUs) per O.D. unit of β -galactosidase per minute. The triplicates are averaged for each concentration and plotted

as normalized RLUs against the dose of agonist or as fold induction vs the dose of agonist. The results of testing with a variety of different compounds are presented in the following table:

Compound	Quantity	Relative light units
None	---	6.2
Androstenol	25 μ M	0.3
Zaragozic acid	37 μ M	7.2
Squelestatin	20 μ M	6.5
Lovastatin	1 μ M	6.2
Compactin	1 μ M	5.8
Aminobenzotriazole	10 μ M	9.3
Indomethacin	100 μ M	7.1
Nordihydroquiaretic acid	50 μ M	4.1
Squalene	10 μ M	6.8
Retinoic acid	10 μ M	5.9
Epiandrostenone + 5 α -pregnenalone	@ 50 μ M	3.4
Phenobarbitol	50 μ M	7.5
Leukotriene B ₄	500 ng/ml	6.8
Prostaglandin E ₂	5 μ g/ml	6.9
Octanoic acid	400 μ M	8.5
<i>t</i> - β -carotene	5 μ M	7.1
Farnesol	50 μ M	10.2
Pregnenalone	50 μ M	8.0
Cholesterol	50 μ M	7.4
Arachidonic acid	30 μ M	4.6
5-Hydroxyeicostetraenoic acid + 15-Hydroxyeicostetraenoic acid(R)	@ 500 ng/ml	7.0
8-Hydroxyeicostetraenoic acid(R,S)	500 ng/ml	8.5
25-OH-cholesterol	10 μ M	5.9

Compound	Quantity	Relative light units
Vitamin K1/K2	@ 2.5 μ M	7.6
reverse triiodothyronine	5 μ M	8.8
Anhydro-retinol	50 μ M	6.8
14-OH-retroretinol	1.4 μ M	8.0
Taurocholic acid + Taurodeoxycholic acid	@ 200 μ M	5.8
Dehydroepiandrostenone	50 μ M	5.9
Vitamin E	50 μ M	6.8

This example demonstrates the androstans (such as androstenol) are effective at reducing the constitutive activity of the CAR isoform employed herein.

5 The selectivity of a modulator for a particular receptor can be measured by comparing the activation/repression of that receptor with the activation/repression of some other related receptor with the same modulator.

Example 4

10 Dose response of CAR or CAR-like isoforms to
modulators therefor

Effector plasmid, reporter plasmid, and β -galactosidase control plasmid are co-transfected into CV-1 cells at a ratio of about 1:3:5, using a liposome-mediated method, employing N-{1-(2,3-dioleoyloxy)propyl-N,N,N-trimethyl ammonium methyl sulfate} (i.e., DOTAP (Boehringer Mannheim) according to manufacturer's instructions in Dulbecco's modified Eagle's medium (DMEM) with 10% delipidated hormone-depleted fetal calf serum.

20 After about 2-3 hours, the cells are washed twice with fresh DMEM and test compound is added to the media to the final molar concentration indicated in Figure 1. After

24-48 hours of incubation, the media is removed and the cells are lysed. Aliquots are assayed for luciferase and β -galactosidase activity. Luciferase activity is normalized to optical density units of β -galactosidase per
5 minute of incubation.

The data are expressed in Figure 1 as the normalized response to solvent or test compound, relative to induction of the same construct incubated in solvent alone.

10 Review of Figure 1 reveals that the androstans (such as androstenol, androstenol-3-acetate, 5 α -androstan-3 α -ol, and the like) are effective at suppressing the constitutive activity of CAR or CAR-like isoforms, with androstenol and 5 α -androstan-3 α -ol being the
15 presently preferred androstans for use in the practice of the present invention.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are
20 within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Evans, Ronald M.
Forman, Barry M.
- (ii) TITLE OF INVENTION: MODULATORS FOR NEW MEMBERS OF THE
STEROID/THYROID SUPERFAMILY OF RECEPTORS
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Pretty, Schroeder, Brueggemann & Clark
 - (B) STREET: 444 South Flower Street, Suite 2000
 - (C) CITY: Los Angeles
 - (D) STATE: CA
 - (E) COUNTRY: USA
 - (F) ZIP: 90071
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/442,464
 - (B) FILING DATE: 16-MAY-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Reiter, Stephen E.
 - (B) REGISTRATION NUMBER: 31,192
 - (C) REFERENCE/DOCKET NUMBER: P41 9881
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 619-546-4737
 - (B) TELEFAX: 619-546-9392

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1450 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 273..1319

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTGAGCTTGC TCCTTAAGTT ACAGGAAGTC TCCTTATAAT AGACACTTCA TTTTCCTAGT	60
CCATCCCTCA TGA AAAAATGA CTGACCACTG CTGGGCAGCA GGAGGGATGA TAATCCTAAC	120
TCCAATCACT GGCAACTCCT GAGATCAGAG GAAAACCAGC AACAGCGTGG GAGTTGGGG	180
AGAGGCATTC CATACCAGAT TCTGTGGCCT GCAGGTGACA TGCTGCCTAA GAGAAGCAGG	240

22

AGTCTGTGAC	AGCCACCCCA	ACACGTGACG	TC	ATG	GCC	AGT	AGG	GAA	GAT	GAG		293				
				Met	Ala	Ser	Arg	Glu	Asp	Glu						
				1				5								
CTG	AGG	AAC	TGT	GTG	GTA	TGT	GGG	GAC	CAA	GCC	ACA	GGC	TAC	CAC	TTT	341
Leu	Arg	Asn	Cys	Val	Val	Cys	Gly	Asp	Gln	Ala	Thr	Gly	Tyr	His	Phe	
		10					15					20				
AAT	GCG	CTG	ACT	TGT	GAG	GGC	TGC	AAG	GGT	TTC	TTC	AGG	AGA	ACA	GTC	389
Asn	Ala	Leu	Thr	Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg	Thr	Val	
		25				30					35					
AGC	AAA	AGC	ATT	GGT	CCC	ACC	TGC	CCC	TTT	GCT	GGA	AGC	TGT	GAA	GTC	437
Ser	Lys	Ser	Ile	Gly	Pro	Thr	Cys	Pro	Phe	Ala	Gly	Ser	Cys	Glu	Val	
	40				45					50					55	
AGC	AAG	ACT	CAG	AGG	CGC	CAC	TGC	CCA	GCC	TGC	AGG	TTG	CAG	AAG	TGC	485
Ser	Lys	Thr	Gln	Arg	Arg	His	Cys	Pro	Ala	Cys	Arg	Leu	Gln	Lys	Cys	
				60					65					70		
TTA	GAT	GCT	GGC	ATG	AGG	AAA	GAC	ATG	ATA	CTG	TCG	GCA	GAA	GCC	CTG	533
Leu	Asp	Ala	Gly	Met	Arg	Lys	Asp	Met	Ile	Leu	Ser	Ala	Glu	Ala	Leu	
			75					80					85			
GCA	TTG	CGG	CGA	GCA	AAG	CAG	GCC	CAG	CGG	CGG	GCA	CAG	CAA	ACA	CCT	581
Ala	Leu	Arg	Arg	Ala	Lys	Gln	Ala	Gln	Arg	Arg	Ala	Gln	Gln	Thr	Pro	
		90				95					100					
GTG	CAA	CTG	AGT	AAG	GAG	CAA	GAA	GAG	CTG	ATC	CGG	ACA	CTC	CTG	GGG	629
Val	Gln	Leu	Ser	Lys	Glu	Gln	Glu	Glu	Leu	Ile	Arg	Thr	Leu	Leu	Gly	
	105					110					115					
GCC	CAC	ACC	CGC	CAC	ATG	GGC	ACC	ATG	TTT	GAA	CAG	TTT	GTG	CAG	TTT	677
Ala	His	Thr	Arg	His	Met	Gly	Thr	Met	Phe	Glu	Gln	Phe	Val	Gln	Phe	
	120				125					130					135	
AGG	CCT	CCA	GCT	CAT	CTG	TTC	ATC	CAT	CAC	CAG	CCC	TTG	CCC	ACC	CTG	725
Arg	Pro	Pro	Ala	His	Leu	Phe	Ile	His	His	Gln	Pro	Leu	Pro	Thr	Leu	
				140					145					150		
GCC	CCT	GTG	CTG	CCT	CTG	GTC	ACA	CAC	TTC	GCA	GAC	ATC	AAC	ACT	TTC	773
Ala	Pro	Val	Leu	Pro	Leu	Val	Thr	His	Phe	Ala	Asp	Ile	Asn	Thr	Phe	
			155					160					165			
ATG	GTA	CTG	CAA	GTC	ATC	AAG	TTT	ACT	AAG	GAC	CTG	CCC	GTC	TTC	CGT	821
Met	Val	Leu	Gln	Val	Ile	Lys	Phe	Thr	Lys	Asp	Leu	Pro	Val	Phe	Arg	
		170				175					180					
TCC	CTG	CCC	ATT	GAA	GAC	CAG	ATC	TCC	CTT	CTC	AAG	GGA	GCA	GCT	GTG	869
Ser	Leu	Pro	Ile	Glu	Asp	Gln	Ile	Ser	Leu	Leu	Lys	Gly	Ala	Ala	Val	
		185				190					195					
GAA	ATC	TGT	CAC	ATC	GTA	CTC	AAT	ACC	ACT	TTC	TGT	CTC	CAA	ACA	CAA	917
Glu	Ile	Cys	His	Ile	Val	Leu	Asn	Thr	Thr	Phe	Cys	Leu	Gln	Thr	Gln	
	200				205					210					215	
AAC	TTC	CTC	TGC	GGG	CCT	CTT	CGC	TAC	ACA	ATT	GAA	GAT	GGA	GCC	CGT	965
Asn	Phe	Leu	Cys	Gly	Pro	Leu	Arg	Tyr	Thr	Ile	Glu	Asp	Gly	Ala	Arg	
			220					225					230			
GTG	GGG	TTC	CAG	GTA	GAG	TTT	TTG	GAG	TTG	CTC	TTT	CAC	TTC	CAT	GGA	1013
Val	Gly	Phe	Gln	Val	Glu	Phe	Leu	Glu	Leu	Leu	Phe	His	Phe	His	Gly	
			235					240					245			
ACA	CTA	CGA	AAA	CTG	CAG	CTC	CAA	GAG	CCT	GAG	TAT	GTG	CTC	TTG	GCT	1061
Thr	Leu	Arg	Lys	Leu	Gln	Leu	Gln	Glu	Pro	Glu	Tyr	Val	Leu	Leu	Ala	
		250					255					260				

23

GCC ATG GCC CTC TTC TCT CCT GAC CGA CCT GGA GTT ACC CAG AGA GAT Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg Asp 265 270 275	1109
GAG ATT GAT CAG CTG CAA GAG GAG ATG GCA CTG ACT CTG CAA AGC TAC Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Thr Leu Gln Ser Tyr 280 285 290 295	1157
ATC AAG GGC CAG CAG CGA AGG CCC CGG GAT CGG TTT CTG TAT GCG AAG Ile Lys Gly Gln Gln Arg Arg Pro Arg Asp Arg Phe Leu Tyr Ala Lys 300 305 310	1205
TTG CTA GGC CTG CTG GCT GAG CTC CGG AGC ATT AAT GAG GCC TAC GGG Leu Leu Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Glu Ala Tyr Gly 315 320 325	1253
TAC CAA ATC CAG CAC ATC CAG GGC CTG TCT GCC ATG ATG CCG CTG CTC Tyr Gln Ile Gln His Ile Gln Gly Leu Ser Ala Met Met Pro Leu Leu 330 335 340	1301
CAG GAG ATC TGC AGC TGAGGCCATG CTCACTTCCT TCCCCAGCTC ACCTGGAACA Gln Glu Ile Cys Ser 345	1356
CCCTGGATAC ACTGGAGTGG GAAAATGCTG GGACCAAAGA TTGGGCCGGG TTCAAAGGGA	1416
GCCCAGTGGT TGCAATGAAA GACTAAAGCA AAAC	1450

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 348 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ser Arg Glu Asp Glu Leu Arg Asn Cys Val Val Cys Gly Asp 1 5 10 15
Gln Ala Thr Gly Tyr His Phe Asn Ala Leu Thr Cys Glu Gly Cys Lys 20 25 30
Gly Phe Phe Arg Arg Thr Val Ser Lys Ser Ile Gly Pro Thr Cys Pro 35 40 45
Phe Ala Gly Ser Cys Glu Val Ser Lys Thr Gln Arg Arg His Cys Pro 50 55 60
Ala Cys Arg Leu Gln Lys Cys Leu Asp Ala Gly Met Arg Lys Asp Met 65 70 75 80
Ile Leu Ser Ala Glu Ala Leu Ala Leu Arg Arg Ala Lys Gln Ala Gln 85 90 95
Arg Arg Ala Gln Gln Thr Pro Val Gln Leu Ser Lys Glu Gln Glu Glu 100 105 110
Leu Ile Arg Thr Leu Leu Gly Ala His Thr Arg His Met Gly Thr Met 115 120 125
Phe Glu Gln Phe Val Gln Phe Arg Pro Pro Ala His Leu Phe Ile His 130 135 140

24

His Gln Pro Leu Pro Thr Leu Ala Pro Val Leu Pro Leu Val Thr His
 145 150 155 160
 Phe Ala Asp Ile Asn Thr Phe Met Val Leu Gln Val Ile Lys Phe Thr
 165 170 175
 Lys Asp Leu Pro Val Phe Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser
 180 185 190
 Leu Leu Lys Gly Ala Ala Val Glu Ile Cys His Ile Val Leu Asn Thr
 195 200 205
 Thr Phe Cys Leu Gln Thr Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr
 210 215 220
 Thr Ile Glu Asp Gly Ala Arg Val Gly Phe Gln Val Glu Phe Leu Glu
 225 230 235 240
 Leu Leu Phe His Phe His Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu
 245 250 255
 Pro Glu Tyr Val Leu Leu Ala Ala Met Ala Leu Phe Ser Pro Asp Arg
 260 265 270
 Pro Gly Val Thr Gln Arg Asp Glu Ile Asp Gln Leu Gln Glu Glu Met
 275 280 285
 Ala Leu Thr Leu Gln Ser Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg
 290 295 300
 Asp Arg Phe Leu Tyr Ala Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg
 305 310 315 320
 Ser Ile Asn Glu Ala Tyr Gly Tyr Gln Ile Gln His Ile Gln Gly Leu
 325 330 335
 Ser Ala Met Met Pro Leu Leu Gln Glu Ile Cys Ser
 340 345

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCYGARGGNT GYAARGGNTC TTT

23

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

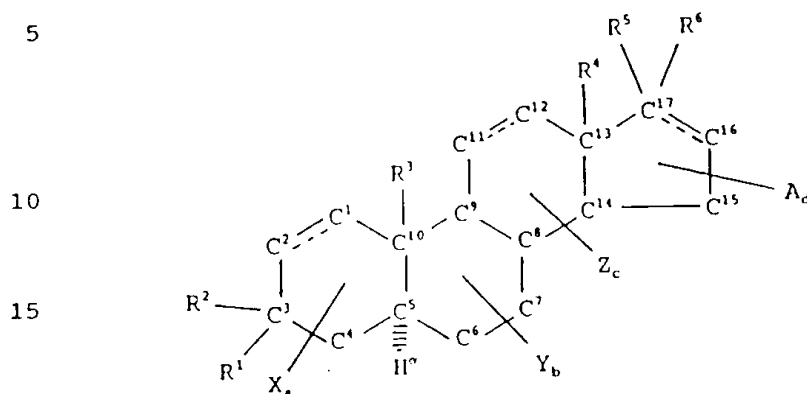
- (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Glu Gly Cys Lys Gly Phe Phe
1 5

That which is claimed is:

1. A method for modulating the activity of an isoform of CAR or a CAR-like species, said method comprising administering an effective amount of a steroid-like compound having the structure:



wherein:

- 20 $R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and
 R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen,
lower alkyl, acyl or trimethylsilyl;
 R^3 and R^4 are each independently hydrogen or lower
alkyl;
25 $R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6
is absent when there is a double bond
between C^{16} and C^{17} ;
 X , Y , Z and A are each independently selected
from hydroxy, alkoxy (of a lower alkyl
30 group), mercapto (of a lower alkyl group),
halogen, trifluoromethyl, cyano, nitro,
amino, carboxyl, carbamate, sulfonyl,
sulfonamide;
a falls in the range of 0 up to 4;
35 b falls in the range of 0 up to 4;
c falls in the range of 0 up to 4; and
d falls in the range of 0 up to 3.

2. A method according to claim 1 wherein said isoform of CAR or CAR-like species is a member of the steroid/thyroid superfamily of receptors having at least 75 % overall amino acid homology with the receptor set forth in SEQ ID NO:1 (CAR- α), at least 88 % amino acid homology in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid homology in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

3. A method according to claim 1 wherein said isoform of CAR or a CAR-like species has at least 75 % overall amino acid identity with the receptor set forth in SEQ ID NO:1 (CAR- α), at least 88 % amino acid identity in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid identity in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

4. A method according to claim 1 wherein said member has at least 86 % overall amino acid similarity with the receptor set forth in SEQ ID NO:1 (CAR- α), at least 91 % amino acid similarity in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 87 % amino acid similarity in the ligand binding domain thereof, with respect to the ligand binding domain of the receptor set forth in SEQ ID NO:1.

5. A method according to claim 1 wherein R^1 is hydrogen and R^2 is α -OR, wherein R is as defined above.

6. A method according to claim 5 wherein R is hydrogen or acyl.

7. A method according to claim 1 wherein R^3 is methyl.

8. A method according to claim 1 wherein R^4 is methyl.

9. A method according to claim 1 wherein $R^5 = R^6 = O$.

10. A method according to claim 1 wherein R^5 and R^6 are both hydrogen.

11. A method according to claim 1 wherein R^6 is absent, and there is a double bond between C^{16} and C^{17} .

12. A method for the identification of compounds which modulate the activity of an isoform of CAR or a CAR-like species, said method comprising:

contacting host cell(s) containing receptor-
5 encoded DNA and a suitable hormone response element linked to reporter-encoded DNA with test compound, and
determining the effect of test compound on the level of expression of said reporter.

13. A method according to claim 12 wherein said isoform of CAR or a CAR-like species is a member of the steroid/thyroid superfamily of receptors having at least 75 % overall amino acid homology with the receptor set forth
5 in SEQ ID NO:1 (CAR- α), at least 88 % amino acid homology in the DNA binding domain thereof, with respect to the DNA binding domain of the receptor set forth in SEQ ID NO:1, and at least 74 % amino acid homology in the ligand binding domain thereof, with respect to the ligand binding domain
10 of the receptor set forth in SEQ ID NO:1.

14. A method according to claim 12 wherein said response element is a direct repeat of two or more half sites separated by a spacer of four or five nucleotides, wherein each half site comprises the sequence

5 N_x-RGBNNM-,

 wherein

 R is selected from A or G;

 B is selected from G, C, or T;

10 each N is independently selected from
 A, T, C, or G;

 M is selected from A or C; and

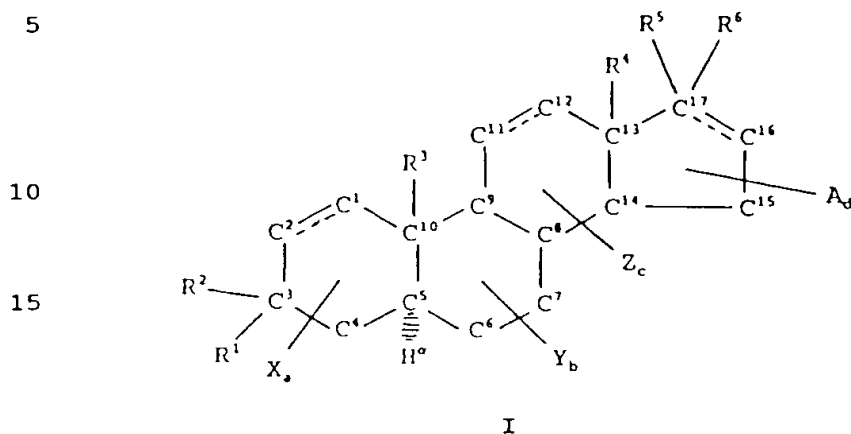
 x falls in the range of 0 up to 5;

 with the proviso that at least 4 nucleotides of
15 said -RGBNNM- sequence are identical with the nucleotides
 at corresponding positions of the sequence -AGGTCA-.

15. A method to increase the libido of a subject, said method comprising modulating the activity of an isoform of CAR or a CAR-like species.

16. A method according to claim 15 wherein said isoform of CAR or a CAR-like species is a member of the steroid/thyroid superfamily of receptors having at least 75 % overall amino acid homology with the receptor set forth
5 in SEQ ID NO:1 (CAR- α), at least 88 % amino acid homology
 in the DNA binding domain thereof, with respect to the DNA
 binding domain of the receptor set forth in SEQ ID NO:1,
 and at least 74 % amino acid homology in the ligand binding
 domain thereof, with respect to the ligand binding domain
10 of the receptor set forth in SEQ ID NO:1.

17. A method to increase the libido of a subject, said method comprising administering to said subject a libido-enhancing amount of a steroid-like compound having the structure I as follows:



20 wherein:

$R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and

R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17} ;

X , Y , Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

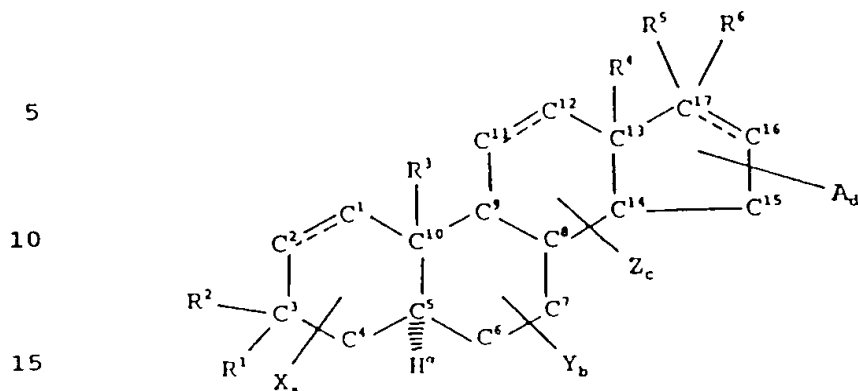
a falls in the range of 0 up to 4;

b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

18. A physiologically active composition comprising a compound having the structure I as follows:



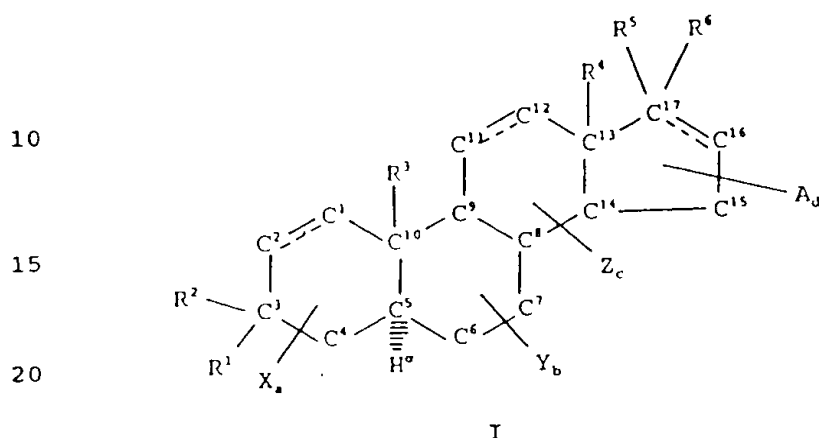
I

wherein:

- 20 $R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and
 R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen,
lower alkyl, acyl or trimethylsilyl;
- R^3 and R^4 are each independently hydrogen or lower
alkyl;
- 25 $R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6
is absent when there is a double bond
between C^{16} and C^{17} ;
- X , Y , Z and A are each independently selected
from hydroxy, alkoxy (of a lower alkyl
group), mercapto (of a lower alkyl group),
30 halogen, trifluoromethyl, cyano, nitro,
amino, carboxyl, carbamate, sulfonyl,
sulfonamide;
- a falls in the range of 0 up to 4;
- b falls in the range of 0 up to 4;
- 35 c falls in the range of 0 up to 4; and
- d falls in the range of 0 up to 3

in a suitable vehicle rendering said compound amenable to
oral, transdermal or nasal delivery.

19. A method for ameliorating the libido-reducing effects of a 5 α -reductase inhibitor, said method comprising co-administering, to a subject being treated with 5 α -reductase inhibitor(s), a libido-enhancing amount of a steroid-like compound having the structure I as follows:



wherein:

$R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and

R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17} ;

X , Y , Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

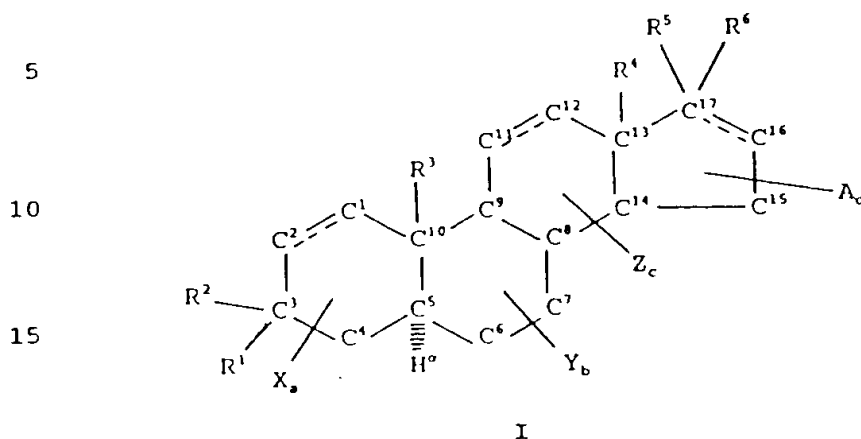
b falls in the range of 0 up to 4;

c falls in the range of 0 up to 4; and

d falls in the range of 0 up to 3.

20. A method according to claim 19 wherein said 5α -reductase inhibitor is finasteride (PROSCAR).

21. A composition comprising a 5α -reductase inhibitor and a libido-enhancing amount of a steroid-like compound having the structure I as follows:



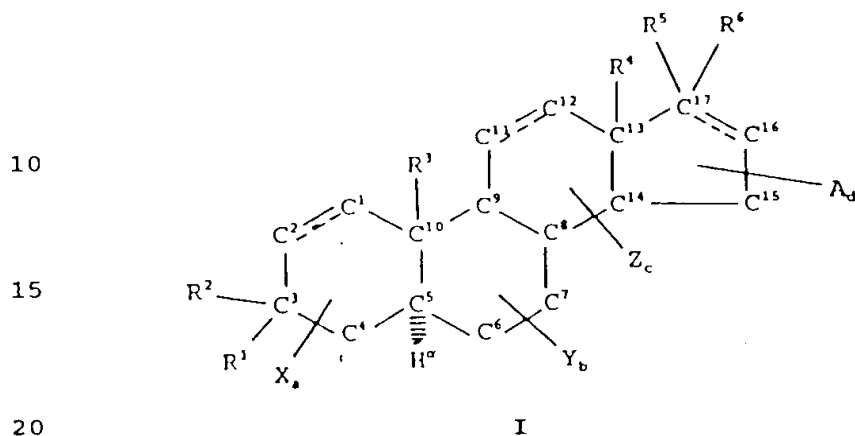
wherein:

- 20 $R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and
 R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen,
lower alkyl, acyl or trimethylsilyl;
 R^3 and R^4 are each independently hydrogen or lower
alkyl;
25 $R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6
is absent when there is a double bond
between C^{16} and C^{17} ;
 X , Y , Z and A are each independently selected
from hydroxy, alkoxy (of a lower alkyl
30 group), mercapto (of a lower alkyl group),
halogen, trifluoromethyl, cyano, nitro,
amino, carboxyl, carbamate, sulfonyl,
sulfonamide;
 a falls in the range of 0 up to 4;
35 b falls in the range of 0 up to 4;
 c falls in the range of 0 up to 4; and
 d falls in the range of 0 up to 3.

22. A composition according to claim 21 wherein said 5 α -reductase inhibitor is finasteride (PROSCAR).

23. Method of screening cells or cell extracts to determine the presence of receptors involved in the modulation of libido, said method comprising

contacting cells or cell extracts with a compound
5 having the structure I as follows:



wherein:

$R^1 = R^2 = O$; or $R^1 = \text{hydrogen}$ and

R^2 is $\alpha\text{-OR}$, wherein R is selected from hydrogen, lower alkyl, acyl or trimethylsilyl;

R^3 and R^4 are each independently hydrogen or lower alkyl;

$R^5 = R^6 = O$; or R^5 and R^6 are both hydrogen; or R^6 is absent when there is a double bond between C^{16} and C^{17} ;

X , Y , Z and A are each independently selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide;

a falls in the range of 0 up to 4;

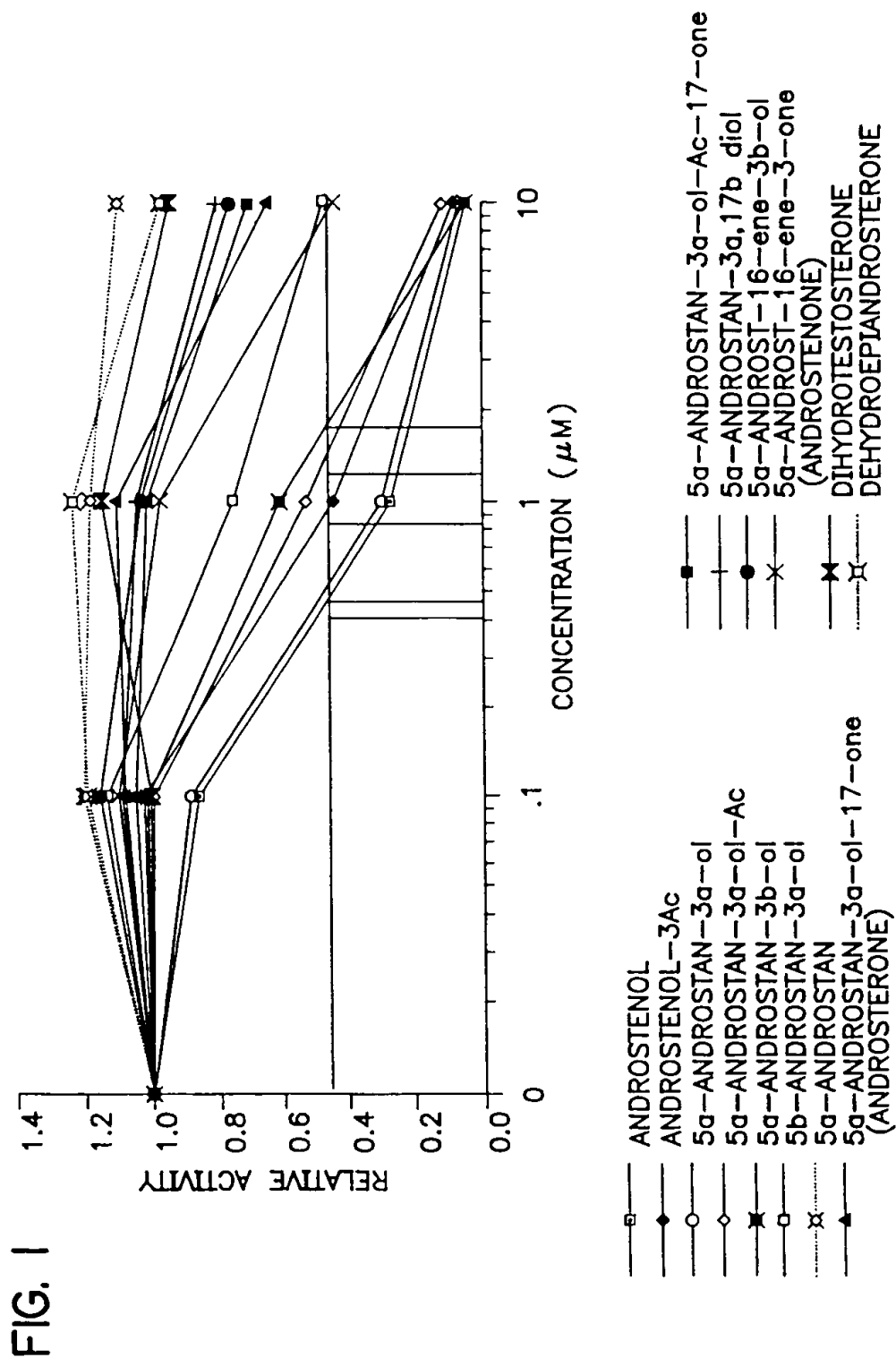
35

b falls in the range of 0 up to 4;
c falls in the range of 0 up to 4; and
d falls in the range of 0 up to 3,

40 and thereafter

identifying those cells or cell extracts which
bind said compound.

1 / 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03865

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : A16K 31/56, 31/365; G01N 33/53, 33/566 US CL : 514/177, 178, 179, 180, 182; 435/7.1, 7.21, 7.8 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/177, 178, 179, 180, 182; 435/7.1, 7.21, 7.8 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, HCAPLUS, REGISTRY. search terms: retino?, stereo?, androst? Moore.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BAES et al. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Molecular and cellular biology. March 1994, Vol. 14, No. 3, pages 1544-1552, see entire document.	1-11
A, P	MANGELSDORF et al. The nuclear receptor superfamily: the second decade. Cell. 15 December 1995, Vol. 83, pages 835-839, see entire document.	1-11
A, P	MANGELSDORF et al. The RXR heterodimers and orphan receptors. Cell. 15 December 1995, Vol. 83, pages 841-850, see entire document.	1-11
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family	
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 02 JULY 1996	Date of mailing of the international search report 27 AUG 1996	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MICHAEL D. PARK <i>Michael Collins for</i>	
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03865

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HEYMAN et al. 9-cis retinoic acid is a high affinity ligand for the retinoic x receptor. Cell. 24 January 1992, Vol. 68, pages 397-406, see entire document.	1-11
A, P	FORMAN et al. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell. 02 June 1995, Vol. 81, pages 687-693, see entire document.	1-11
X	BENNUA-SKALMOWSKI et al. A facile conversion of primary or secondary alcohols with n-perfluorobutane-sulfonyl fluoride/1,8-diazabicyclo[5.4.0]undec-7-ene into their corresponding fluorides. Tetrahedron letters. 10 April 1995, Vol. 36, pages 2611-2614, see entire document.	18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03865

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11, 18

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03865

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-11, 18 drawn to a method for modulating the activity of an isoform of CAR and a physiological active composition.

Group II: Claims 12-14 drawn to a method for the identification of compounds which modulate the activity of an isoform of CAR.

Group III: Claims 15-16 drawn to a method to increase the libido of a subject, said method comprising modulating the activity of an isoform of CAR.

Group IV: Claim 17 drawn to a method to increase the libido of a subject, said method comprising administering to said subject a libido-enhancing amount of a steroid-like compound.

Group V: Claims 19-20 a method for ameliorating the libido-reducing effects of a 5-alpha-reductase.

Group VI: Claims 21-22 drawn to a composition of 5-alpha-reductase inhibitor and a libido-enhancing amount of a steroid like compound.

Group VII: Claim 23 drawn to a method of screening cells.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons. Group I is drawn to a method for modulating the activity of an isoform of CAR and a physiological active composition comprising a compound having the structure I. The special technical feature of Group I is the method of modulating the activity of an isoform of CAR or a CAR-like species by administering an effective amount of a steroid like compound. Group VI is drawn to another composition of 5-alpha-reductase inhibitor and a libido-enhancing amount of a steroid like compound. Group VI special technical feature is the 5-alpha-reductase inhibitor and is different from group I. Groups II-V, and VII are drawn to different methods of using the receptor or the steroid like compound structure I which do not share the same or corresponding special technical feature as group I. Note that PCT Rule 13 does not provide for multiple products or methods within a single application. Since the special technical feature of each group invention is not present in any other group invention, unity of invention is lacking.